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Studies on Simultaneous Determination of Acetaminophen, Salicylic Acid and Salicyluric Acid in Biological Fluids by High Performance Liquid Chromatography

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Summary: A rapid and sensitive high performance liquid chromatographic method was developed for separation and quantitation of acetaminophen, salicylic acid and salicyluric acid in human plasma and urine. The method involved ethyl acetate extraction of the three drugs from plasma or urine followed by evaporation of the organic solvent phase and dissolution of the residue in 100 µl methanol. A 20 µl aliquot was analysed on a reversed phase column using an isocratic system of 60 ml/l acetonitrile in 4 mmol/l phosphate buffer, pH 5.7 as a mobile phase and a variable wave length UV detector set at 237 nm. The procedure was used to determine the amounts of the three compounds in plasma and urine of two healthy volunteers who ingested 650 mg of aspirin® followed one hour later by 650 mg of acetaminophen.

Untersuchungen zur simultanen Bestimmung von Acetaminophen, Salicylsäure und Salicylursäure in biologischen Flüssigkeiten mit Hochleistungsflüssigchromatographie

Zusammenfassung: Zur Trennung und quantitativen Bestimmung von Acetaminophen, Salicylsäure und Salicylursäure im menschlichen Plasma und Harn wurde eine schnelle und empfindliche hochleistungsflüssigchromatographische Methode entwickelt. Die drei Pharmaka werden mit Ethylacetat aus Plasma oder Harn extrahiert, das Lösungsmittel abgedunstet und der Rückstand in 100 µl Methanol gelöst. Ein Aliquot von 20 µl wird an einer „reversed phase"-Säule mit einem isokratischen System von 60 ml/l Acetonitril in 4 mmol/l Phosphatpuffer pH 5,7 als mobiler Phase und einem UV-Detektor (λ = 237 nm) analysiert.

Das Verfahren wurde zur Bestimmung der drei Verbindungen in Plasma und Harn zweier gesunder Probanden, die 650 mg Aspirin® und eine Stunde später 650 mg Acetaminophen eingenommen hatten, eingesetzt.

Introduction

Acetaminophen and salicylates are the most common drugs used as analgesics and antipyretics. Although these are supposed to be given singly, there are many commercial products available which contain both in a single dosage form. In order to achieve a rapid therapeutic effect without the risk of chronic salicylism, physicians prescribe these drugs in a combined, alternate dosage regimen.

Alteration of the mode of biotransformation of acetaminophen by other drugs has been well documented. *Houston & Levy* (1) demonstrated that ascorbic acid, administered in conjunction with acetaminophen, significantly reduces the excretion rate of acetaminophen sulfate. *Levy* et al. (2) demonstrated that salicylamide competitively inhibits the glucuronidation and sulfate conjugation of acetaminophen.

We hypothise that co-administration of acetaminophen and salicylates, especially for prolonged periods, may cause competitive inhibition of glucuronidation of acetaminophen. Saturation of this pathway may result in shunting to other pathways with formation of toxic metabolites of acetaminophen which may lead to hepatocellular death (3).

This potential hazard from combination of both drugs prompted us to develop a simple and rapid method for separation and quantitation of both drugs and their metabolites in biological fluids.

A number of spectrophotometric (5–7) methods used for detection and quantitation of these drugs, can detect only one drug when both are present. Also, they are insensitive and time consuming. Gas-liquid chromatographic methods have been used for determination and quantitation of acetaminophen (8, 9), and salicylates (10, 11). These methods are sensitive and specific, how-

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ever, chemical derivatisation is necessary. Hence, they are time consuming and require high technical experience.

High performance liquid chromatographic methods (12–14) were used for determination of acetaminophen alone. Other HPLC methods (15–19) were used for determination of salicylic acid and/or salicyluric acid. There is only one HPLC method (20) that determined both acetaminophen and salicylic acid simultaneously. However, the close and short retention times of acetaminophen and salicylic acid (2.15 and 2.90 min respectively) prevented the complete separation of both compounds from each other and from other biological compounds.

We describe in this report a reliable method for simultaneous quantitative determination of acetaminophen, salicylic acid and salicyluric acid in plasma or urine using HPLC.

Materials and Methods

Apparatus

A liquid chromatographic system (Waters Associates, Inc., Milford, Massachusetts 01757) consisted of models 6000A and M45 pumps, model 720 system controller, model 730 data module and an automatic sample injector model 710B "WISP" (Waters Intelligent Sample Processor). The effluent was monitored by UV variable wave length detector (Beckman model 155, Berkeley, California 94710). Separation was accomplished using a Waters μ Bondapak C18 column, 3.9 mm i.d. × 30 cm containing 10 μm particles of octadecylsilane-bonded spherical silica. Water and solvents were filtered with Gelman 0.2 μm filters and degassed under vaccum prior to use.

Reagents

Acetaminophen (4-acetamidophenol, 98%), sodium salicylate (99%) and salicyluric acid (*o*-hydroxyhippuric acid, 97%) were purchased from Aldrich Chemical Company, Inc., (Milwaukee, Wisconsin 53233). Ethyl acetate (HPLC grade) was obtained from Fisher Scientific Company (Fair Lawn, New Jersey 07410). Acetonitrile (HPLC grade) was purchased from Burdic & Jackson Laboratories, Inc., (Muskegon, Michigan 49442). Other chemicals and reagents were of the highest purity available and were obtained from various commercial sources.

Analytical procedures

Extraction

100 μl of plasma or urine was diluted to 1.0 ml with distilled water after adjusting pH of plasma to 2.0 and pH of urine to 5.0. Fifteen ml of ethyl acetate and 4 g sodium chloride were added to the plasma or urine in 40 ml volume glass tube with a glass stopper. The tube was shaken vigorously on a shaking apparatus for 15 min then centrifuged for 10 min to separate the phases. Ten ml of the ethyl acetate phase were aspirated into a clean tube. The ethyl acetate was evaporated to dryness at 45 °C under a gentle stream of nitrogen. The residue was dissolved in 100 μl methanol and mixed thoroughly using a vortex. A 20 μl aliquot was injected into the liquid chromatograph.

HPLC analysis

After several trials, optimal separation of the three compounds was accomplished by eluting with 60 ml/l acetonitrile in 4 mmol/l phosphate buffer, pH 5.7. The flow rate was set at 1 ml/min. The module chart speed was 1 cm/min. Absorbance was monitored at 237 nm and recorded on the data module. Quantitation of peak areas using external standard technique was carried out using the data module integrator. Internal standard was found unnecessary, because by repeated injections of the three compounds into HPLC, reproducible results to within 4% were constantly achieved, besides the simplicity and complete recovery of the extraction procedure.

Standard curve of actaminophen, salicylic acid and salicyluric acid in plasma and urine

Stock solution was prepared by dissolving 30 mg of each of acetaminophen, salicylic acid and salicyluric acid in 100 ml distilled water. Working standards were prepared by making appropriate dilutions of the stock standard solution.

Different concentrations of each of the three compounds ranging from 5 mg/l to 50 mg/l were added to drug-free plasma and urine. Extraction for each sample was carried out as mentioned before. A standard curve was obtained by plotting the concentration of each sample against the peak area.

Urinary excretion of free acetaminophen, salicylic acid and salicyluric acid in man

Two healthy volunteers, 26 and 33 years old and weighing 163 and 136 pounds (ca. 74 and ca. 62 kg) and with no history of drug intake in the twelve week period preceding the test were studied. Each volunteer was given an oral dose of 650 mg of aspirin® after an overnight fast. One hour later, each subject was given 650 mg of acetaminophen. No food was allowed up to two hours after acetaminophen intake. Both volunteers were allowed to drink water and fruit juices to increase urine flow. Urine samples were collected from aspirin® administration every 1 to 2 hours up to 12 hours, and at 24 hours. Urine samples were filtered and pH and volume of each sample were determined. The samples were stored at – 30 °C up till the time of analysis.

Plasma concentration of free acetaminophen, salicylic acid and salicyluric acid in man

Blood samples were taken every 15 min to 1 hour up to four hours following drug administration. Each blood sample was received in a heparinized test tube and centrifuged. Plasma was aspirated and stored at – 30 °C up till the time of analysis.

Results and Discussion

The objective of this report is to develop a simple and sensitive HPLC method for simultaneous separation and quantitation of acetaminophen, salicylic acid and its main metabolite salicyluric acid in biological fluids. Also, to evaluate previously reported methodology for these compounds (10, 12–19). The method of *Miceli* et al. (20), which was carried out only on plasma for simultaneous measurement of acetaminophen and salicylic acid lacks the complete separation of both compounds due to their close retention times (2.15 and 2.90 min). The short retention times of both compounds prevent their clear separation from the biological background when this procedure is applied to urine samples. In addition, this method did not describe salicyluric acid determination. The method developed in our laboratory is simple, sensitive and can be applied for determination of both compounds and their metabolites in serum or urine in subtherapeutic, therapeutic or toxic doses of each or both drugs.

Optimization of extraction efficiency

Extraction solvent

Several solvents have been used for extraction of acetaminophen, salicylic acid and salicyluric acid. *Levy & Yamada* (2) and others (12, 13) used diethyl ether for extraction of acetaminophen from plasma and urine while *Rowland & Riegelman* (10) used the same solvent for extraction of salicylic acid from plasma. *Levy & Procknal* (21) used ethylene dichloride for extraction of both salicylic acid and salicyluric acid from urine samples. *Miceli* et al. (20) used a solvent mixture of chloroform and isopropanol 1/1 by volume for extraction of both acetaminophen and salicylic acid from serum. In an attempt to develop one method of extraction of the three compounds from plasma and urine simultaneously, we tried these solvents in addition to ethyl acetate. Data in table 1 show the extraction efficiency of these solvents to 10 mg/l of each of acetaminophen, salicylic acid and salicyluric acid added to drug-free plasma and urine. The same extraction procedure was applied for all solvent systems used.

From plasma the extraction efficiency of ether for acetaminophen and salicyluric acid was found to be high, however, for salicylic acid it was low. This indicates that ether is not a good extraction solvent for salicylic acid contrary to an earlier report (10). Ethylene dichloride has a very low extraction efficiency for acetaminophen and it was found that its capability for extraction of both salicylic acid and salicyluric acid lies within 50–55%. This may seem contradictory to the previously reported methods considering ethylene dichloride as an efficient extractor for these compounds. This can be explained by the difference in pH of the biological medium during extraction. Ethylene dichloride was used previously as an extraction solvent at very low pH (21) which allows more efficient extraction for salicylic acid and salicyluric acid. This low pH may be suitable for spectrophotometric methods but we observed in our HPLC analysis that at this low pH other compounds were extracted and interfered with the compounds under study. The extraction efficiency of ethyl acetate from plasma at pH 2 was found to be high for the three compounds without interference by any of the biological compounds. Chloroform/isopropanol, 1/1 by volume has a high extraction efficiency for acetaminophen, moderate for salicylic acid and low for salicyluric acid at this pH (tab. 1).

From urine ether can extract acetaminophen with a high efficiency at pH 5 but it has a low extraction efficiency for salicylic acid at this pH. Ethylene dichloride has a low extraction efficiency at pH 5 for the three compounds. Ethyl acetate was found to have the highest extraction efficiency for these three compounds. Extraction from urine at pH 5 was found unreliable for chloroform/isopropanol mixture due to interference by other compounds extracted with the test compounds. As shown in table 1, the extraction efficiency of ethyl acetate for the three compounds from plasma at pH 2 ranges from 98 to 104%. Recovery of acetaminophen, salicylic acid and salicyluric acid from urine at pH 5 was 98%, 87% and 87% respectively.

Effect of pH of the biological medium

The pH of the biological medium affects to a great extent the solvent's extraction efficiency for these compounds. Our study indicates that salicylic acid and salicyluric acid are better extracted at a more acidic pH. This is due to the minimum ionization of salicylic acid at a pH below its pK_a (3.0). Acetaminophen extraction was found not to be greatly affected by changes in pH. Data in table 2 show the extraction efficiency of ethyl acetate for the three compounds at pH 2 and pH 5. As shown the three compounds are completely extracted from plasma at pH 2. Extraction at this pH can only be applied to plasma due to no interference from biological background. However, in urine, as shown in figure 1, extraction at pH 2 is unreliable due to the interference by other compounds simultaneously extracted along with these compounds. Repeated extractions of known concentrations of the three compounds added to drug-

Tab. 1. Extraction efficiency (recovery, %, $\bar{x} \pm s$) of various solvent systems for acetaminophen, salicylic acid and salicyluric acid from plasma (extraction at pH 2)) and urine (extraction at pH 5).
* values obtained are unreliable due to interference by biological background. ** less than 1% recovery.

	Recovery (%)							
	Ether		Ethylene dichloride		Chloroform/Isopropanol 1 + 1		Ethyl acetate	
	Plasma	Urine	Plasma	Urine	Plasma	Urine	Plasma	Urine
Acetaminophen (10 mg/l)	99 ± 4	96 ± 3	15 ± 4	17 ± 4	93.7 ± 4	*	98 ± 2	98 ± 4
Salicylic acid (10 mg/l)	34.7 ± 5	41.3 ± 2	55 ± 2	5.3 ± 2	65.8 ± 6	*	98 ± 2	87 ± 4
Salicyluric acid (10 mg/l)	107 ± 6	91 ± 4	52.4 ± 3	**	48.2 ± 6	*	104 ± 6	87 ± 6

Tab. 2. Effect of pH on the extraction efficiency of ethyl acetate for acetaminophen, salicylic acid and salicyluric acid from plasma. Data shown are mean values ± SD.

	Drug added to plasma (mg/l)	Amount extracted at pH 2 (mg/l)	Extraction efficiency (%)	Amount extracted at pH 5 (mg/l)	Extraction efficiency (%)
Acetaminophen	10	9.8 ± 0.2	98 ± 2	9.8 ± 0.6	98 ± 6
Salicylic acid	10	9.8 ± 0.2	98 ± 2	8.7 ± 0.3	87 ± 3
Salicyluric acid	10	10.4 ± 0.6	104 ± 6	8.7 ± 0.4	87 ± 4

free urine at different pH indicated that at pH 5 extraction efficiency ranged from 87 to 98% for all compounds studied. Minimal interference by other biological materials was observed at this pH. Interestingly, *Miceli* et al. reported (20) that they extracted acetaminophen and salicylic acid at plasma pH (7.4). At that pH salicylic acid is highly ionized and hence very weakly soluble in organic solvents.

Optimization of the HPLC system condition

Effect of mobile phase

Different mobile phases have been reported for the separation and quantitation of these compounds individually or in combination. When we tried one of these mobile phases as (methanol 9 g/l acetic acid; fractions 0.12/0.88) (22), a constant peak was observed having the same retention time as acetaminophen in the blank biological samples. Hence, it interfered with acetaminophen determination. Under this condition, salicylic acid and salicyluric acid have long retention times (tab. 3).

Tab. 3. Effect of mobile phase on the retention times of acetaminophen, salicylic acid and salicyluric acid.

Mobile phase		Retention time (min)		
Solvent (A)	Solvent (B)	Salicylic acid	Salicyluric acid	Acetaminophen
Acetonitrile 400 ml	2 g/l Acetic acid 600 ml	6.7	4.7	3.4
Methanol 120 ml	9 g/l Acetic acid 880 ml	32.7	23.3	8.2
Acetonitrile 70 ml	4 mmol/l Phosphate buffer 930 ml	5.3	6.2	8.4
Acetonitrile 60 ml	4 mmol/l Phosphate buffer 940 ml	5.7	7.4	9.7

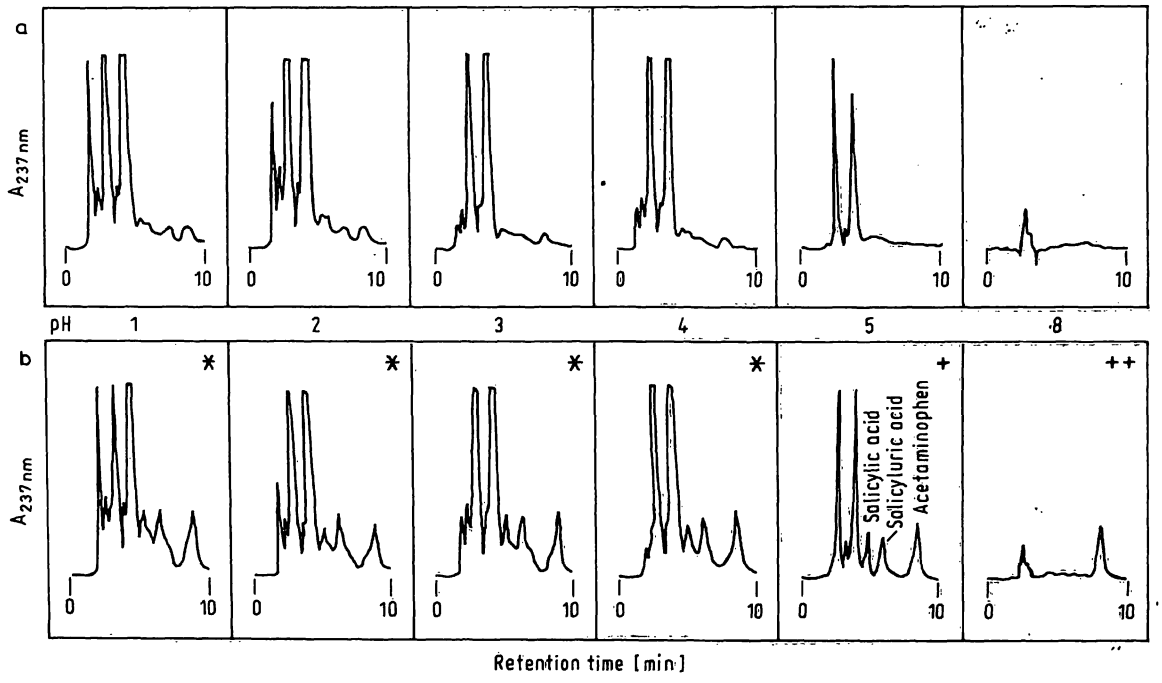

Fig. 1. Effect of pH on the extraction efficiency of ethyl acetate for acetaminophen, salicylic acid and salicyluric acid from urine.
a) Blank urine extract b) Extract of urine, spiked with 10 mg/l each of acetaminophen, salicylic acid and salicyluric acid.
* values obtained are unreliable due to interference by other biological compounds.
+ extraction efficiencies for acetaminophen, salicylic acid and salicyluric acid are 98 ± 2, 87 ± 4 and 87 ± 4 respectively.
++ extraction efficiency for acetaminophen 97 ± 3, but for salicylic acid and salicyluric acid are less than 1%.

Using other mobile phases resulted in short retention times for all compounds and overlapping by biological background. For these reasons we tried to find a new mobile phase that could clearly separate these compounds without interference from other biological background. We found that 60 ml/l acetonitrile in 4 mmol/l phosphate buffer is an ideal mobile phase for this study. Data in table 3 illustrate different mobile phases used and the corresponding retention times of the three compounds studied.

Effect of pH of mobile phase

The retention time was found to be affected to a great extent by the change in pH of mobile phase. It was observed that the lower the pH the longer the retention times of the three compounds studied. Increasing the pH resulted in short retention times. Data in table 4 indicate that pH 5.7 is optimal for separation of the three compounds. Slight alteration such as decimal increase or decrease in the pH was found to alter the retention times. Alteration of pH not only affects the retention time but also changes the time intervals between the individual compounds. Therefore separation is affected.

Tab. 4. Effect of pH of mobile phase (acetonitrile, 60 ml/l in 4 mmol/l phosphate buffer) on the retention times of acetaminophen, salicylic acid and salicyluric acid.

pH of mobile phase	Retention time (min)		
	Salicylic acid	Salicyluric acid	Acetaminophen
5.7	5.7	7.4	9.7
5.0	7.8	10.9	11.2
7.0	4.3	4.9	9.8

Effect of UV detector wave length

The previous HPLC systems used different wave lengths for monitoring acetaminophen, salicylic acid or salicyluric acid. A wave length of 300 nm was used (14, 19) for monitoring salicylic acid. Wave lengths ranging from 247 nm to 254 nm were used (12, 13, 20, 22) for monitoring acetaminophen or salicylic acid or both. A wave length 237 nm was used (16) for monitoring both salicylic acid and salicyluric acid. In an attempt to find the UV wave length which provided maximum sensitivity for these compounds, we compared the absorbance of each compound separately and in combination with the other two compounds at various wave lengths. The data in table 5 indicate that 237 nm is the most suitable wave length for monitoring the three compounds simultaneously. For acetaminophen the highest absorbance was observed at wave lengths 237 nm and 250 nm, while the highest absorbances for salicylic acid and salicyluric acid were observed at 237 nm. Hence, those previous methods which monitored these last two compounds at wave lengths other than 237 nm lacked the maximum sensitivity and probably underestimated these compounds especially at low concentrations.

The retention times for salicylic acid, salicyluric acid and acetaminophen were 5.7, 7.4 and 9.7 min respectively. Figure 2a shows a chromatogram of a human plasma blank at pH 2 after extraction by ethyl acetate. Figure 2b shows a chromatogram of a human plasma blank spiked with 10 µg each of acetaminophen, salicylic acid and salicyluric acid and extracted as described before. Figure 2c shows a chromatogram of a human plasma sample 1.5 h after 650 mg of aspirin and 0.5 h

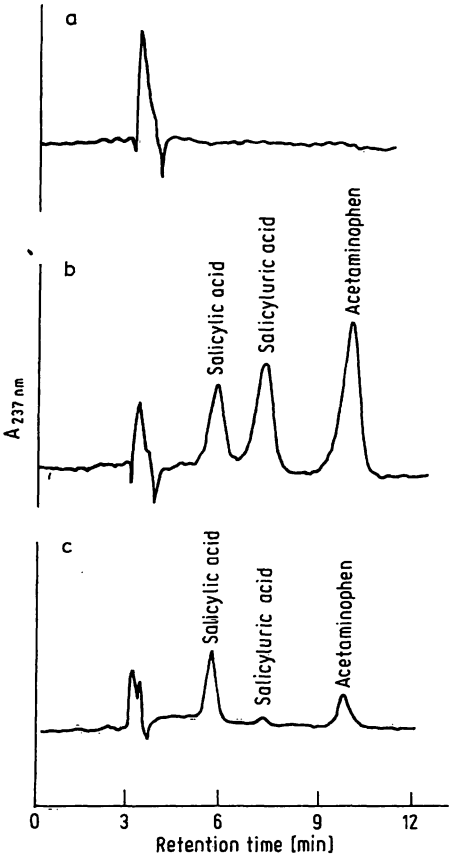

Fig. 2. High performance liquid chromatogram of human plasma extract: (a) blank plasma, (b) plasma spiked with 10 mg/l each of salicylic acid, salicyluric acid and acetaminophen and (c) human plasma sample 1.5 hour after 650 mg aspirin® and 0.5 hour after the same dose of acetaminophen.

Tab. 5. Absorbance and peak areas of 10 mg/l each of acetaminophen, salicylic acid and salicyluric acid at different wave lengths.

(nm)	Acetaminophen		Salicylic acid		Salicyluric acid	
	Absorbance	Peak area	Absorbance	Peak area	Absorbance	Peak area
237	0.025	4294	0.014	2050	0.016	2473
250	0.027	4309	0.004	311	0.012	1934
300	0.003	119	0.009	1159	0.008	1152

after the same dose for acetaminophen. Figure 3a shows a chromatogram of a human urine blank extracted at pH 5. Figure 3b illustrates the same standards added to drug-free urine and extracted. Figure 3c shows a chromatogram of a human urine sample 2 h after 650 mg each of aspirin and acetaminophen. As shown, chromatograms from blank plasma yielded an extraneous peak around 3.5 min, while chromatograms from blank urine produce peaks between 3.5 and 4.7 min. None of these peaks showed any interference with the three compounds under study.

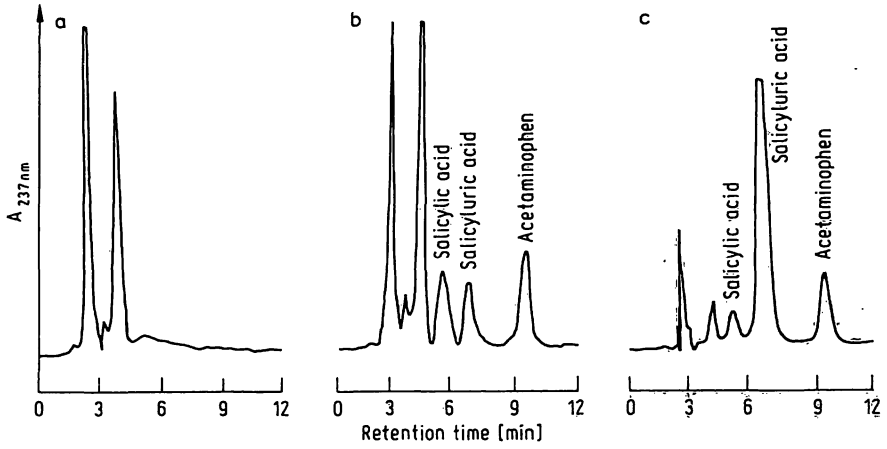

Fig. 3. High performance liquid chromatogram of human urine extract: (a) blank urine, (b) urine spiked with 10 mg/l each of salicylic acid, salicyluric acid and acetaminophen and (c) human urine sample 2.0 hours after 650 mg of aspirin® and 1.0 hour after the same dose of acetaminophen.

Accuracy and precision of this method for acetaminophen, salicylic acid and salicyluric acid in human plasma and urine are shown in tables 6 and 7. The three compounds were added to drug-free plasma and urine in concentrations ranging from 5 to 50 mg/l each and extracted as described before. The standard curves show a linear relationship between the concentrations and peak areas over these ranges. The coefficient of variation of these results is less than 4% in all concentrations studied. Concentrations lower or higher than this

Tab. 6. Recovery of acetaminophen, salicylic acid and salicyluric acid after extraction from plasma at pH 2. Coefficient of variation is less than 4% over these concentrations.

Drug added	N	Acetaminophen		Salicylic acid		Salicyluric acid	
		Drug recovery	Recovery	Drug recovery	Recovery	Drug recovery	Recovery
(mg/l)		(mg/l)	(%), $\bar{x} \pm s$	(mg/l)	(%), $\bar{x} \pm s$	(mg/l)	(%), $\bar{x} \pm s$
5	10	4.8	96 ± 3.2	4.7	94 ± 2.8	4.8	96 ± 1.8
10	10	9.8	98 ± 0.65	9.8	98 ± 0.88	10.4	104 ± 0.72
20	10	19.6	98 ± 1.2	19	95 ± 0.92	20.2	101 ± 1.2
30	10	28.8	96 ± 0.89	28.8	96 ± 2.2	28.9	96.4 ± 0.72
40	10	41.6	104 ± 1.5	39.96	99.9 ± 1.8	40.4	101 ± 2.2
50	10	48	96 ± 2.4	48.2	96.3 ± 2.7	51.5	103 ± 1.8

Tab. 7. Recovery of acetaminophen, salicylic acid and salicyluric acid after extraction from urine at pH 5. Coefficient of variation is less than 4% over these concentrations.

Drug added	N	Acetaminophen		Salicylic acid		Salicyluric acid	
		Drug recovered	Recovery	Drug recovered	Recovery	Drug recovered	Recovery
(mg/l)		(mg/l)	(%), $\bar{x} \pm s$	(mg/l)	(%), $\bar{x} \pm s$	(mg/l)	(%), $\bar{x} \pm s$
5	10	4.9	98 ± 2.8	4.4	87 ± 3.2	4.45	89 ± 2.8
10	10	9.8	98 ± 0.74	8.8	88 ± 1.2	8.8	88 ± 0.92
20	10	19.5	97.6 ± 1.8	17.6	88 ± 2.2	17.4	87 ± 1.8
30	10	28.9	96.3 ± 1.2	25.5	85 ± 1.8	26.1	87 ± 1.2
40	10	39.8	99.5 ± 1.9	34.8	87 ± 1.2	34.4	86 ± 1.2
50	10	47	94 ± 2.8	43	86 ± 2.1	43	86 ± 1.9

range can be estimated with the same efficiency by changing the sample or methanol volumes or by changing the UV detector sensitivity.

This method was used to study the urinary excretion in 24 h of free acetaminophen, salicylic acid and salicyluric acid in two healthy human volunteers who ingested 650 mg of aspirin® followed 1 h later by 650 mg of acetaminophen. Figure 4 illustrates the excretion of the three compounds in g/l at various times. As shown, acetaminophen concentration in urine was at its highest level 1–2 hours after drug administration. Salicylic acid and salicyluric acid reached their highest concentration level within 3 to 4 hours following drug administration. From this study we observed that the amount of free acetaminophen excreted in 24 hours accounts for 3.6 to 4.5% of acetaminophen dose. Salicylic acid accounts for 6.2 to 9.5% of the aspirin® dose while salicyluric acid excretion accounts for 59 to 60% of the aspirin® dose. These figures agree with other reports (5, 23).

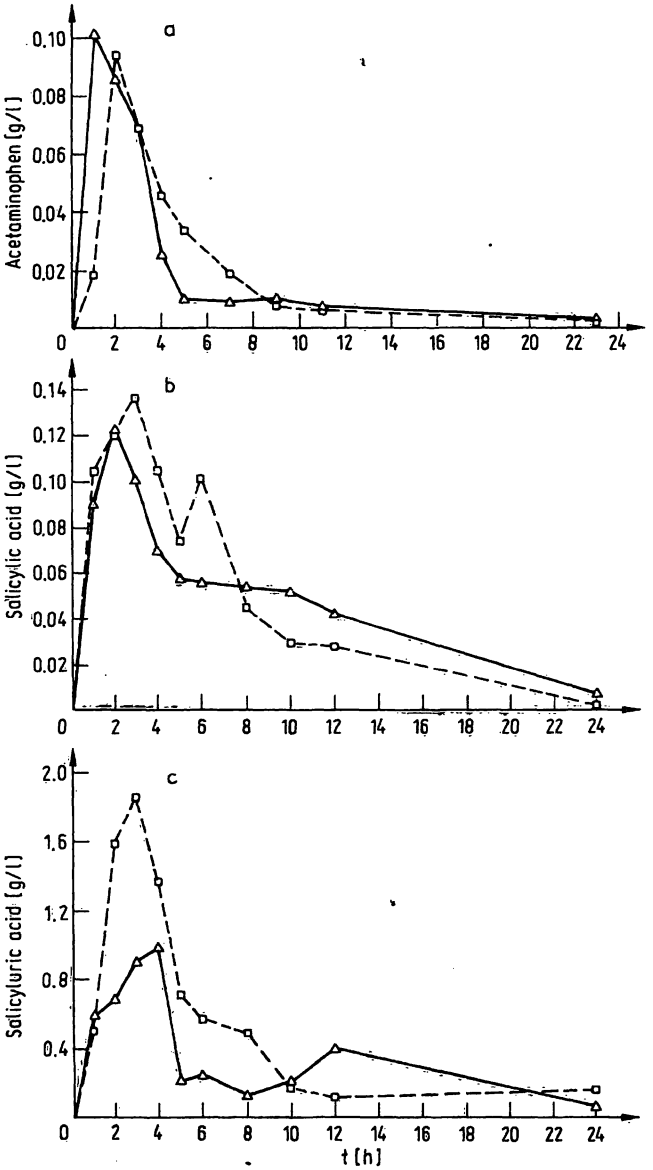

Fig. 4. Twenty-four hour excretion of acetaminophen (a), salicylic acid (b) and salicyluric acid (c) in two human subjects after a single dose (650 mg) each of aspirin® and acetaminophen.

This method was also used to study the plasma concentrations of the three compounds for the same volunteers who ingested the previous doses of both aspirin® and acetaminophen. Blood samples were collected every 15 min to 1 hour up to 4 hours following drug administration. Figure 5 illustrates the plasma concentration versus time of each compound. As shown, the highest plasma concentration of acetaminophen which is 11 mg/l was obtained 0.5 hour after drug administration. Salicylic acid and salicyluric acid have their highest plasma concentrations (35 mg/l and 2.8 mg/l respectively) about 1.5 hour after oral aspirin® administration. The plasma concentrations of the three compounds over 4 hours in our study are in agreement with results presented by *Levy* et al. (24).

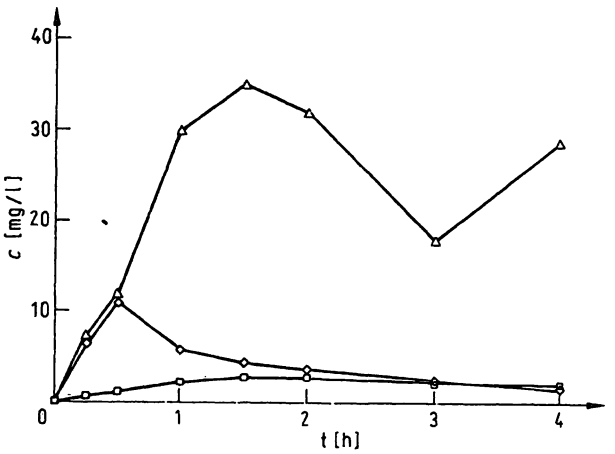

Fig. 5. Plasma concentration of, salicylic acid △—△, salicyluric acid ◇—◇ and acetaminophen □—□ over four hours after a single dose (650 mg) each of aspirin® and acetaminophen.

In summary, this HPLC method is simple and reliable for determination and quantitation of acetaminophen, salicylic acid and salicyluric acid in biological fluids. This method is highly sensitive and can detect and quantitate each of the three compounds in a concentration up to 1 mg/l. The three compounds can be extracted by one solvent and separated during one run in HPLC. A single run lasts only for 15 minutes. This method can also be used for determination of other salicylates and acetaminophen metabolites after acid or enzymatic hydrolysis of these conjugates to the free compound (2, 21). We are currently using this method for pharmacokinetic and pharmacodynamic studies of a combination of acetaminophen and salicylates in human.

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